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Chapter

Noncoding RNAs in Myelodysplastic Syndromes

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Abstract

The discovery of short regulatory RNAs has recently directed the attention of scientists to parts of the genome that previously had been regarded as "junk" DNA because they did not encode protein products. The revelation that even protein-noncoding sequences had biological functions began the era of discovering the world of noncoding RNAs (ncRNAs). Of these ncRNAs, microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) are the most numerous and best-known ncRNA groups. miRNAs and lncRNAs are important regulators of hematopoiesis, and their abnormal function has serious implications for phenotypes. Deregulation of these ncRNAs is found in hematopoietic disorders, and they also contribute to the development and progression of myelodysplastic syndromes (MDS). Properties of ncRNAs such as stability and tissue specificity make these molecules highly promising diagnostic and prognostic markers as well as interesting therapeutic targets. This chapter summarizes our knowledge on the contribution of ncRNAs to the pathogenesis of MDS and discusses their potential applicability in disease diagnostics and prognosis.

Keywords: myelodysplastic syndromes, noncoding RNAs, microRNAs, long noncoding RNAs, pathogenesis

1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell (HSC) disorders, characterized by dysplastic and ineffective blood cell production, with a tendency for transformation to acute myeloid leukemia (AML). The diagnosis is generally suspected based on the presence of an abnormal complete blood count and is confirmed by performing bone marrow (BM) aspiration and biopsy, demonstrating morphological evidence of dysplasia. A number of additional tests, including cytogenetics, flow cytometry, and molecular genetics, are needed to complete the laboratory evaluation of patients with MDS [1].

Because of the large heterogeneity of MDS, the development of additional molecular tools able to refine the prognostic scoring system, to predict outcome, and to monitor the response to treatment is required. Recently, application of new high-throughput methods such as next-generation sequencing (NGS) has identified recurrent somatic mutations in MDS cells. In particular, point mutations in the TP53, EZH2, ETV6, RUNX1, and ASXL1 genes have been shown to be associated

with specific clinical features and poor overall survival, independent of established risk factors [2]. Even though approximately 78% of MDS patients carry at least one oncogenic mutation [3], there is a long list of mutations in more than 50 genes with often unclear etiology, complicating the use of somatic mutations as simple and universal markers of MDS prognosis.

Concerning MDS pathogenesis, substantial progress has been made in recent years. A vast literature has become available regarding the spectrum of cytogenetic abnormalities, gene mutations, epigenetic modifications, gene expression patterns, and deregulated signaling pathways (e.g., apoptosis, proliferation, immune response, chromatin remodeling, RNA-splicing machinery, oxidative damage/DNA repair, microenvironment interactions, and others) associated with the disease. In this review, we discuss the contributions of noncoding RNAs (ncRNAs) to the pathogenesis of MDS as well as their potential applications as novel molecular markers for clinical purposes.

2. Noncoding RNAs

At the end of the last millennium, the importance of noncoding RNAs was completely unknown. Up to that point, the scientific community focused on genes that coded for proteins. The classic dogma of molecular biology postulated that DNA was transcribed into RNA, which was then translated into protein, ignoring all non-protein-coding sequences. Only in 1993 did the importance of miRNAs begin to be revealed. The discovery of the first miRNA, lin-4, from *Caenorhabditis elegans* [4, 5] initiated a new scientific era that definitively overcame the absolute sanctity of the central dogma. Interest in this field was further stimulated by the finding that almost all of the mammalian genome was transcribed at some level [6], raising speculation that much of this pervasive transcription was likely functional. This idea was epitomized by the ENCODE (Encyclopedia of DNA Elements, www.encodeproject.org) consortium that claimed to have assigned "biochemical functions for 80% of the genome" [7, 8]. From the beginning of this era, researchers identified thousands of previously unknown types of noncoding RNAs and indeed started to reveal their multiple functions affecting various features of cells.

2.1 Types of noncoding RNAs

Functional ncRNAs can be divided into two main types: infrastructural and regulatory ncRNAs. Infrastructural ncRNAs appear to have housekeeping roles in translation and splicing; they include species such as ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and small nuclear RNAs (snRNAs) that are involved in splicing events. Regulatory ncRNAs, including long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs) are involved in the modification and interactions with other RNAs. However, ncRNAs can also be categorized on the basis of length (small, 18–31 nt; medium, 31–200 nt; and long, >200 nt), structure (circular RNAs (circRNAs)), or subcellular localization (small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs)). Other infrequent ncRNA types such as trans-spliced transcripts and macroRNAs that comprise enormous genomic distances, or multigene transcripts containing several genes or even the whole chromosome, further complicate efforts at systematic classification [9]. Moreover, clear categorization of ncRNA species is difficult, as many ncRNA transcripts often share properties of several categories. The major classes of ncRNAs are summarized in **Table 1**.

ncRNA class	Description	Description	Length
rRNA	Ribosomal RNA	RNA that is directly incorporated into the ribosome	Large subunit (5S–121 nt, 5.8S–156 nt, 28S–5070 nt) Small subunit (18S–1869 nt)
tRNA	Transfer RNA	Transfer amino acids to the ribosome for protein construction	76–90 nt
snRNA	Small nuclear RNA	Small RNA located in the nucleus, involved in spliceosomes (e.g., U1, U2, U5, U4, and U6), RNA modification, and other functions. Also commonly referred to as U-RNAs	~150 nt
snoRNA	Small nucleolar RNA	RNA located in the nucleolus, mostly involved in modification of other RNAs, such as rRNA (C/D-box and H/ACA box snoRNAs) or spliceosomal RNA (scaRNA)	~60–250 nt
miRNA	microRNA	A short single-stranded RNA that usually suppresses the translation of target mRNA by binding to 3′ UTR through RNA interference pathways	21–25 nt
siRNA	Small interfering RNA	Double-stranded RNA that guides sequence-specific degradation of target mRNA through RNA interference pathway	10–25 bp
piRNA	Piwi-interacting RNA	A large class of small ncRNAs involved in retrotransposon silencing through interactions with piwi proteins	26–31 nt
Y-RNA	Y RNA	Components of the Ro ribonucleoprotein complex, repressing its activity. It is also required for DNA replication	69–112 nt
circRNA	Circular RNA	RNA derived from precursor mRNAs forming covalently closed continuous loop. It is more resistant to exonuclease-mediated degradation	1–5 exons
lncRNA	Long noncoding RNA	A non–protein-coding transcript with >200 nt	>200 nt

Table 1.
The major categories of ncRNAs.

Of the various classes of ncRNAs, miRNAs and lncRNAs are the most numerous and are probably the best-known ncRNA groups (numbers of publications concerning miRNAs and lncRNAs are shown in **Figure 1**). Having different regulatory functions, these types of ncRNAs are important players in the majority of cellular processes, including hematopoiesis, and their abnormal function has serious implications for phenotypes. Deregulation of miRNAs and lncRNAs is frequently found in hematopoietic disorders, contributing substantially to the disease development and progression. Therefore, the following sections of this review will primarily focus on these two categories of ncRNAs and their functions in MDS.

3. MicroRNAs

miRNAs are short single-stranded noncoding RNA molecules of approximately 21–25 nt in length. Their sequences are highly conserved in both plants and animals and are thought to be an evolutionarily ancient component of gene regulation. miRNAs posttranscriptionally regulate gene expression through the RNA

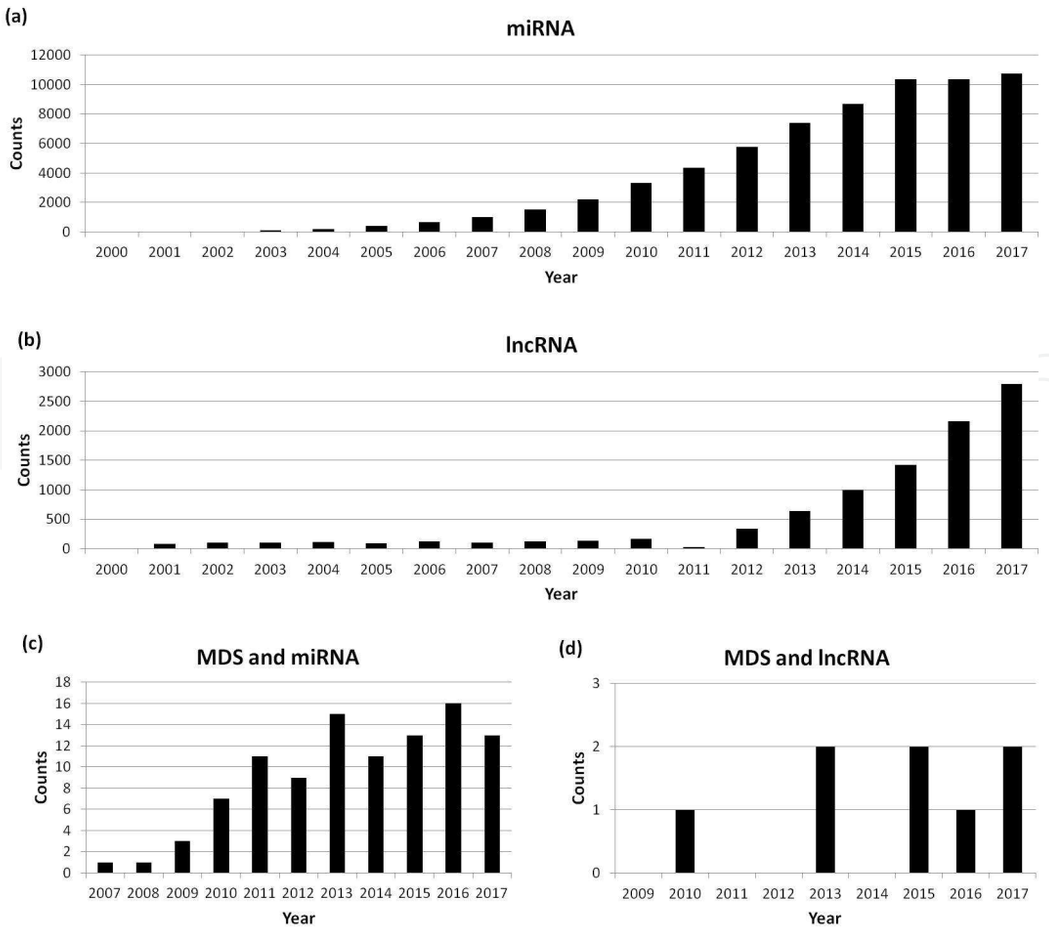

Figure 1.
Numbers of publications found on Pubmed using search terms: (a) miRNA (b) lncRNA, (c) MDS and miRNA, and (d) MDS and lncRNA (since the year 2000).

interference pathway. During the last decade, it has repeatedly been proven that miRNAs play crucial roles in a wide variety of biological processes such as development, differentiation, proliferation, and apoptosis. Because miRNAs influence the expression of genes involved in fundamental signaling pathways, their deregulation often triggers various pathological processes, including cardiovascular diseases, neurological diseases, and cancer.

The first miRNA, lin-4 from *Caenorhabditis elegans*, was discovered in the early 1990s [4, 5]. However, miRNAs were not recognized as a distinct class of biological regulators until the early 2000s. To date, thousands of miRNAs have been identified in humans and other species, and miRNA online sequence repositories, such as the miRBase database (www.mirbase.org), are available. According to the current version of the miRBase database (release 22), there are 1982 precursor miRNAs and 2693 mature miRNAs known in humans.

The biogenesis of miRNAs is a multistep process. miRNA genes are transcribed from genomic DNA by RNA polymerase II, resulting in primary miRNA (pri-miRNA) transcripts that usually encode sequences for several miRNAs. In the nucleus, these pri-miRNAs are cleaved by endonuclease Drosha, releasing approximately 70 nucleotide-long hairpin precursor miRNAs (pre-miRNAs). Pre-miRNAs are transported into the cytoplasm, where they are cleaved by Dicer into dsRNA duplexes containing both mature miRNA strand (miRNA) and its complementary strand (miRNA*). In general, the mature miRNA strands are preferentially loaded into the miRNA-induced silencing complex (miRISC), whereas the complementary strands are excluded and degraded. Once processed from the hairpin and loaded

into the silencing complex, the miRNA pairs with messenger RNA (mRNA) to direct posttranscriptional repression. At sites with extensive pairing complementarity, the miRNA directs argonaute-catalyzed mRNA cleavage. More commonly, however, the miRNAs direct translational repression, mRNA destabilization, or a combination of the two [10].

It has been shown that an individual miRNA is able to control the expression of more than one target mRNA and that each mRNA may be regulated by several miRNAs. Generally, it is believed that miRNAs regulate more than 30% of protein-coding genes in the human genome [11]. The ability of miRNAs to interact with thousands of mRNAs has raised intensive interest in their role in physiological and pathological conditions. Like mRNAs, the majority of miRNAs are expressed in tissue-specific manners. For example, miR-122 is preferentially expressed in liver [12], miR-124 in neurological tissues [13], miR-133 in muscles [14], and miR-208a in heart [15]. Moreover, it has been demonstrated that changes in the spectrum of tissue miRNAs correlate with various pathophysiological conditions [16].

3.1 Extracellular miRNAs

In recent years, cell-free circulating miRNAs have been found in various body fluids such as blood, cerebrospinal fluid, saliva, and urine [17]. The first extracellular small RNAs were observed in blood in 2004 [18]. Unlike the comprehensively described function of cellular miRNAs, the function of miRNAs present in the extracellular environment remains somewhat speculative. However, a growing body of evidence has suggested that these molecules are not mere leftovers of cellular degradation without any specific functions, but that active exchange of miRNAs between cells can play an important role in long-distance cell-to-cell communication.

In 2008, Mitchell et al. [19] reported that extracellular miRNAs were stable in human plasma/serum. This high stability of circulating miRNAs despite high levels of RNase activity in blood indicates that circulating miRNAs must somehow be protected from degradation. To date, a number of miRNA carriers have been described: membrane-derived vesicles (shedding vesicles, exosomes), lipoproteins, and ribonucleoprotein complexes (with argonaute-2 (AGO2) or nucleophosmin 1 (NPM1) proteins) have been found to transport extracellular miRNAs. It has been reported that the sorting of miRNAs into various types of vesicles can be selective. Diehl et al. [20] compared the content of miRNAs in microvesicles and their maternal cells. These authors demonstrated a significantly varied spectrum of miRNAs in both samples, suggesting a selective packaging of miRNAs into microvesicles.

3.2 miRNAs in normal hematopoiesis

The differentiation and homeostasis of the hematopoietic system requires complex and interconnected molecular networks that need careful regulation. During the last decade, the role of miRNAs in the hematopoietic system has been extensively studied, and many miRNAs serving as critical regulators of both normal immune functions and diseases have been discovered (**Figure 2**).

The first study of a role of miRNAs in the differentiation of the immune system showed that forced expression of miR-181 in hematopoietic stem cells (HSCs) markedly increased the number of B lymphocytes, with a concomitant reduction of T lymphocytes [21]. Since then, other miRNAs specific for the maintenance of the "stemness" of HSCs and for the development of individual blood cell lines have been determined. For example, miR-125a was found to be sufficient as a single miRNA to

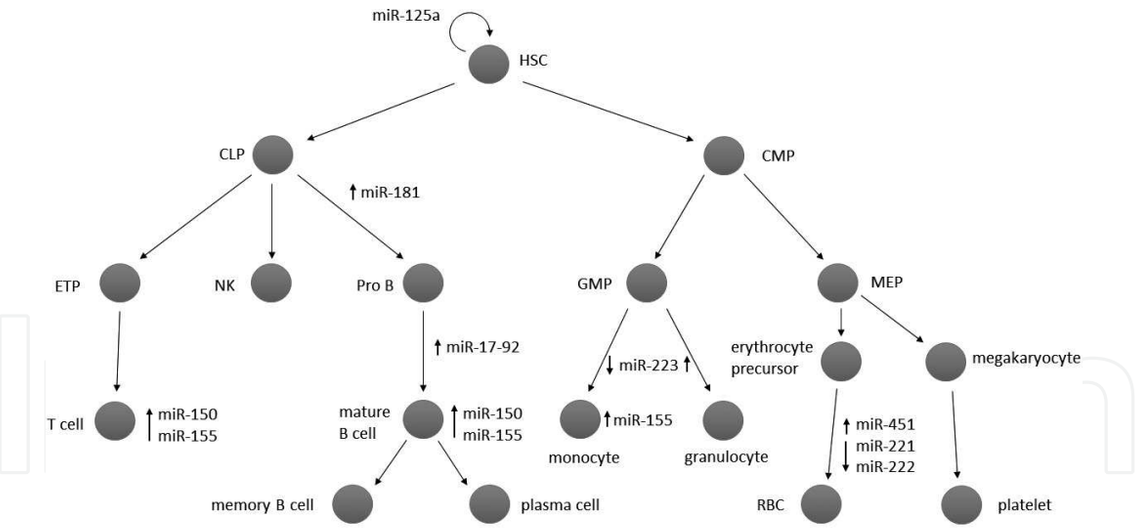

Figure 2.
Schema of lineage differentiation in hematopoiesis and miRNAs involved in the process. CLP—common lymphoid progenitor, CMP—common myeloid progenitor, ETP—early thymic progenitor, GMP—granulocyte macrophage progenitor, HSC—hematopoietic stem cell, MEP—megakaryocyte erythroid progenitor, NK—natural killer cell, RBC—red blood cell.

modulate HSC self-renewal and numbers and to protect lineage-negative progenitor cells from apoptosis [22]. A key miRNA that regulates granulocytic differentiation and function is miR-223. This miRNA shows a highly lineage-specific pattern of expression with low levels in HSCs and common myeloid progenitors. The expression of this miRNA is steadily upregulated during differentiation to granulocytes and is repressed during differentiation to the alternative monocytic fate [23]. miR-451 is expressed predominantly in erythroid cells, and its expression is significantly increased during maturation of erythrocytes. In contrast, miR-221 and miR-222 are downregulated during erythroid differentiation. This downregulation enables the expression of their target gene KIT (KIT proto-oncogene receptor tyrosine kinase, CD117), whose activation triggers erythroblast expansion [24]. Another miRNA, miR-150, is highly expressed in mature lymphocytes, whereas it is not active in HSCs. The target gene of miR-150 is transcription factor MYB (myeloblastosis) that regulates lymphocyte development [25]. miR-155, with its high levels in activated B cells, T cells, and monocytes, also participates in lymphoid differentiation. The development of B cells is positively regulated by miRNAs encoded by cluster miR-17-92. This cluster of miRNAs inhibits the expression of the apoptotic gene BIM (proapoptotic BH3-only Bcl-2 family member) and thus plays a key role in pro-B cells to pre-B cells transition [26]. In our laboratory, we analyzed miRNA expression in individual cell types from the peripheral blood of healthy individuals and determined a panel of 13 miRNAs whose expression profile enables differentiation of individual blood cell lines and determination of the cellular origin of in vitro cultured lines [27].

3.3 miRNAs in malignant hematopoiesis

In oncogenesis, miRNAs act both as oncogenes and as tumor suppressors. Mechanisms of deregulation are similar to those of protein-coding genes (chromosome aberrations, mutations, and epigenetic modifications). In 2002, the loss of two miRNAs (miR-15a and miR-16-1) due to a deletion in the 13q14 region in patients with chronic lymphocytic leukemia was described for the first time as directly associated with malignant disease [28]. In subsequent years, several miRNAs with key roles in the pathogenesis and prognosis of hematological malignancies were detected.

Several publications involved investigations of the role of miRNAs in AML. miRNA expression profiling revealed marked differences in miRNA expression between common cytogenetic subtypes of AML. Jongen-Lavrencic et al. [29] identified upregulation of several miRNAs (miR-382, miR-134, miR-376a, miR-127, miR-299-5p, and miR-323) in AML patients with t(15;17) and significant downregulation of miRNAs from let-7 family in AML with t(8;21) as well as in AML with inv(16). Moreover, miRNA signatures have been reported to be associated with recurrent molecular abnormalities in cytogenetically normal AML [30]. For example, upregulation of miR-10, let-7, and miR-29 family members and downregulation of miR-204 and miR-128a were found in AML with *NPM1* mutations [31] and high expression of miR-181a and miR-181b was associated with *CEBP*A (CCAAT/enhancer binding protein alpha) mutations [32].

3.4 miRNA deregulation in MDS

Several preliminary reports focused on identification of miRNA expression profiles that were either common in MDS or specific for individual MDS subcategories. For example, Pons et al. [33] measured levels of expression of 25 mature miRNAs in mononuclear cells (MNCs) of MDS patients. The authors reported overexpression of miRNA cluster miR-17-92 in MDS and differential expression of miR-15a and miR-16 between low- and high-risk subgroups of patients. Hussein et al. [34] showed that the miRNA profiles in BM cells discriminated MDS with chromosomal alterations from patients with normal karyotypes. Sokol et al. [35] examined miRNA signature in BM MNCs and found deregulation of several miRNAs (increase of miR-222 and miR-10a; decrease of miR-146a, miR-150, and let-7e) in MDS. In our study, we analyzed miRNA expression on a genome-wide level in $CD34^+$ BM cells. We observed significant differences in miRNA expression between early and advanced MDS; an apparent changeover was found between MDS with excess blast 1 (MDS-EB1) and MDS-EB2 subtypes. In particular, we identified strong upregulation of proapoptotic miR-34a in early subtypes of MDS [36].

Although many studies were conducted regarding miRNA profiling in MDS, there have been very few overlaps among them. This inconsistency may mirror the heterogeneity of the disease but also may possibly be explained by variations between the protocols and platforms used for miRNA detection.

There are several lines of evidence that many miRNAs are deregulated in MDS; however, the functions of miRNAs in MDS pathogenesis remain rather unknown. Identification of target genes of miRNAs in MDS and their functional proofs in both in vitro cell cultures and in vivo animal models are necessary to realizing this goal.

3.4.1 miRNAs in MDS with del(5q)

One of the best-characterized MDS subtypes is MDS with isolated del(5q), formerly referred to as 5q– syndrome. Haploinsufficiency of specific genes within common deleted region (CDR) localized in 5q31.3-5q33 locus is essential for the specific phenotype of MDS with del(5q). In addition to protein-coding genes, 13 genes encoding miRNAs are located in CDR. Most importantly, Starczynowski et al. [37] correlated del(5q) haploinsufficiency with loss of two miRNAs that are abundant in hematopoietic stem/progenitor cells (HSPCs), miR-145 and miR-146a. Knockdown of miR-145 and miR-146a together in mouse HSPCs resulted in thrombocytosis, mild neutropenia, and megakaryocytic dysplasia [37].

Kumar et al. [38] showed that miR-145 loss in MDS with del(5q) affects megakaryocyte and erythroid differentiation. These authors found that miR-145 functions through the repression of FLI1 (Friend Leukemia Integration 1 Transcription

Factor), a megakaryocyte and erythroid regulatory transcription factor. Inhibition of miR-145 increases the production of megakaryocytic cells relative to that of erythroid cells. Moreover, the authors proved that combined loss of miR-145 and RPS14 (a ribosomal gene that is required for the maturation of 40S ribosomal subunits and that maps to the CDR) cooperates to alter erythroid-megakaryocytic differentiation in a manner similar to that of the 5q– syndrome [38].

In our studies, we detected high expression of miR-34a in MDS del(5q) patients [36, 39]. The expression of miR-34a is induced by p53, activating apoptosis through inhibition of BCL2 gene (B-Cell CLL/Lymphoma 2, Apoptosis Regulator). This result is consistent with the increased apoptosis of progenitor cells seen in MDS del(5q).

3.4.2 miRNAs related to prognosis of MDS

Several publications have focused on miRNA expression in MDS with regard to prognostic potential. The earliest study in this area associated miRNA profiles with the International Prognostic Scoring System (IPSS) score [35]. A unique signature consisting of 10 miRNAs was closely associated with IPSS risk category permitting discrimination between lower- and higher-risk disease. Selective overexpression of miR-181 family members was detected in higher risk MDS, indicating pathogenetic overlap with AML. Survival analysis revealed shorter survival in patients with high expression of miR-181 family than in patients with low miR-181 expression [35].

Another miRNA that has been identified as having prognostic value in MDS is miR-22 [40]. This miRNA was upregulated in MDS and its level correlated with poor survival. Transgenic mice expressing miR-22 in the hematopoietic cells displayed reduced levels of global 5-hydroxymethylcytosine and increased HSC self-renewal accompanied by defective differentiation. Over time, these mice developed MDS. Interestingly, TET2 gene (Ten-Eleven Translocation 2, Tet Methylcytosine Dioxygenase 2) was identified as a key target of miR-22 in this context [40]. TET2 is a major regulator of DNA demethylation by conversion of methylated cytosine into 5-hydroxymethylcytosine.

3.4.3 miRNAs in the treatment of MDS

Expression profiles of miRNAs also appear to be applicable predictors of treatment responses. Lenalidomide is an immunomodulatory and antiangiogenic agent used for the treatment of MDS with del(5q). In recent years, several studies have analyzed miRNA expression levels before and after lenalidomide treatment in these patients [41–44]. Oliva et al. [41] investigated expression of selected genes/miRNAs at baseline and after 3 and 6 months of lenalidomide treatment. These authors showed that the expression levels of miR-145, miR-146, and miR-155 gradually increased during the course of the treatment. The significant role of miR-143 and miR-145 in response to lenalidomide was confirmed by Venner et al. [42], who showed that lenalidomide selectively abrogated progenitor activity in cells depleted of miR-143 and miR-145, supporting their key role in the sensitivity to lenalidomide in MDS with del(5q). In our studies, the most significant changes in expression levels (decreases) after lenalidomide treatment showed miR-34a and several miRNAs clustered within the 14q32 locus [43, 44]. However, a question remains as to whether the changes in expression levels were due to direct response to lenalidomide or whether they were caused by a reduction of the pathological clone.

Therapy with hypomethylating agents (HMAs) such as azacitidine and decitabine is currently considered to be the standard therapy for higher-risk MDS

and AML with myelodysplasia-related changes. Several studies investigated miRNA expression with respect to HMA treatment in AML [45–47]. Blum et al. [45] proposed miR-29b as a predictive factor for the stratification of older AML patients treated with decitabine; however, this was not confirmed by other studies [46]. Butrym et al. [47] showed that low expression of miR-181 at diagnosis was a predictor of complete remission and prolonged survival in a subset of older AML patients treated with azacitidine.

In relation to HMA therapy in MDS, it was found that the level of extracellular miR-21 was associated with overall response rate and progression-free survival [48]. Furthermore, reduced expression of miR-124 (caused by abnormal methylation) was found in MDS/AML patients responding to decitabine. These patients exhibited significantly lower expression levels of the CDK6 gene (cyclin-dependent kinase 6) that is the target of miR-124 [49]. Moreover, hypermethylation of miR-124-3p gene appeared to be a good prognostic marker of overall survival [50]. In our report, we found that the overall response rate to azacitidine treatment was significantly higher in MDS/AML patients with upregulated miR-17-3p and downregulated miR-100-5p and miR-133b and that the high level of miR-100-5p was associated with shorter overall survival [51].

3.4.4 Extracellular miRNAs in MDS

To date, only a few studies have investigated circulating miRNAs in MDS [48, 52–54]. Two papers [48, 52] focused on specific circulating miRNAs (miR-21, let-7a, and miR-16) that were preselected based on information regarding their deregulation in blood cells and their importance in similar diseases. Researchers monitored the plasma/serum levels of these miRNAs and showed that their levels could serve as prognostic markers for MDS. Kim et al. [48] showed that serum level of miR-21 was significantly associated with overall response rate and progression-free survival in MDS patients treated with HMAs. The publication that studied let-7a and miR-16 demonstrated that high plasma levels of these miRNAs can serve as semi-invasive markers of poor outcome for MDS patients [52].

Zuo et al. [53] measured expression of 800 human miRNAs in MDS plasma. These authors identified a 7-miRNA signature (let-7a, miR-144, miR-16, miR-25, miR-451, miR-651, and miR-655) as an independent predictor of survival in MDS patients with normal karyotypes.

In our study, we investigated the spectrum (2006 human miRNAs) of circulating miRNAs in the plasma of MDS patients [54]. With regard to prognosis, the levels of miR-27a-3p, miR-150-5p, miR-199a-5p, miR-223-3p, and miR-451a were lower in higher risk MDS. Moreover, miR-451a was an independent predictor of progression-free survival, and there was a significant association of miR-223-3p with overall survival [54].

These pioneer studies suggested that plasma levels of specific miRNAs were associated with MDS patient outcome and may add information beyond the currently used scoring systems. Despite these early promising results, there remain insufficient data regarding the full spectrum of extracellular RNAs in MDS. To date, the possible presence of various forms of small noncoding RNAs (apart from mature miRNAs), pathways for their protection, and identification of their cells of origin have not been explored in MDS. These missing information would expand the knowledge regarding extracellular RNAs in this disease, and beyond that, it would definitely contribute to better interpretation of alterations of individual miRNAs with the potential to become specific prognostic markers in MDS.

4. Long noncoding RNAs

lncRNAs form perhaps the most numerous group of ncRNAs. These RNAs are defined as protein-noncoding transcripts longer than 200 nucleotides. This length was proposed to distinguish lncRNAs from small noncoding RNAs. In contrast with protein-coding RNAs, lncRNAs contain only short open reading frames or completely lack them. This group of ncRNAs is characterized by high levels of structural and functional diversity, low levels of GC nucleotides, and lower expression levels, in contrast with protein-coding transcripts. lncRNAs are transcribed by RNA polymerase II or III and subsequently can be spliced and polyadenylated at the 3′ end or may contain a 5′ cap, depending on their biogenesis. Their expression is developmental and tissue-specific.

Some lncRNAs regulate (negatively or positively) the expression of genes in *trans* or *cis* by affecting RNA polymerase II recruitment or by inducing chromatin remodeling. In addition, antisense transcripts can pair with their specific sense RNA, facilitating alternative splicing. When lncRNAs interact with proteins, they can influence protein activity or localization or even help to form cellular substructures or ribonucleoprotein complexes. lncRNAs can be processed to yield small, single- or double-stranded RNAs that can act as endogenous small interfering RNAs (siRNAs) or miRNAs. Moreover, they can also act as "miRNA sponges" that affect the competitive endogenous RNA (ceRNA) network. However, additional functions and detailed signaling pathways of lncRNAs remain to be clarified [55].

According to their position relative to protein-coding mRNAs, lncRNAs are further subcategorized into several groups. Long intergenic noncoding RNAs (lincRNAs) are lncRNAs that are located between annotated protein-coding genes and that are at least 1 kb away from the nearest protein-coding genes. Intronic lncRNAs are coded within introns of protein-coding genes. Sense and antisense lncRNAs are transcribed from the sense or antisense strands of protein-coding genes and often contain exons of this gene with mutual overlap. Bidirectional lncRNAs are oriented head-to-head with protein-coding genes within 1 kb. Bidirectional lncRNA transcripts often exhibit similar expression patterns to those of their protein-coding counterpart, suggesting that they may be subject to shared regulatory pressures. Another group of lncRNA is TERRA (Telomeric Repeat-Containing RNA), transcribed from constitutive heterochromatin-rich regions, or T-UCR (Transcribed Ultraconserved Regions), transcribed from highly conserved regions of the genome.

Although more than 100,000 lncRNAs have been identified to date, only a small number of them have been characterized in detail. To integrate the data describing various lncRNAs, their expression profiles, molecular features, and functions in a variety of cell systems, several databases containing a comprehensive list of lncRNAs have been developed and are continually being updated. Among the most comprehensive databases are LNCipedia (compendium of human lncRNAs, lncipedia.org) [56], lncRNAdb (reference database for functional lncRNAs, lncRNAdb.org) [57], and NRED (database of lncRNA expression, nred.matticklab.com) [58].

4.1 lncRNAs in normal hematopoiesis

Recently, lncRNAs have emerged as important regulators of cell fate. These RNAs play a variety of roles in controlling various steps in hematopoietic differentiation, including maintenance of HSCs and differentiation of myeloid, erythroid, and lymphoid lineages. To date, there have been several descriptions of lncRNAs

crucial for correct function of the hematopoietic system (**Table 2**). The first hematopoiesis-associated lncRNA, EGOT (Eosinophil Granule Ontogeny Transcript), was described in 2007. EGOT, a conserved transcript localized antisense to ITPR1 (Inositol 1,4,5-Trisphosphate Receptor Type 1) that modulates the development of eosinophils, is normally expressed in human $CD34^+$ HSCs, and its expression level increases during eosinophil development, helping to regulate production of eosinophil granule proteins [59].

The relatively well-known lncRNA, H19, maintains quiescence of adult HSCs. The H19 transcript was in fact the first lncRNA to be identified, enriched in the embryonic fetal liver but downregulated after birth [60]. H19 is active in long-term HSCs and becomes gradually downregulated in short-term HSCs and multipotent progenitors. Deletion of H19 from the maternal allele resulted in increased HSC activation and proliferation as well as impaired repopulating ability. This effect is mediated by derepression of maternal IGF2 (Insulin-Like Growth Factor 2) expression and by increased IGF1R (IGF1 Receptor) translation, resulting in increased signaling through the IGF1R [61].

Zhang et al. [62] identified HOTAIRM1 (Homeobox Antisense Intergenic RNA Myeloid 1), which is encoded within the human HOXA (Homeobox A) gene cluster and plays a role in the differentiation of myeloid cells. The expression of this transcript is upregulated during granulocyte differentiation. Knockdown of HOTAIRM1 reduced transcription of HOXA1 and HOXA4 in an acute promyelocytic leukemia cell line, resulting in a decreased expression of genes associated with granulocyte activation, defense response, and maturation [62].

LncRNA	Localization	Function	Reference
EGOT (Eosinophil Granule Ontogeny Transcript)	3p26.1, antisense to ITPR1 gene	Modulates development of eosinophils	[59]
H19	11p15.5, antisense to IGF2	Maintains quiescence of adult HSCs	[60]
HOTAIRM (Hox Antisense Intergenic RNA Myeloid 1)	7p15.2, encoded within the HOXA gene cluster	Induces differentiation of myeloid cells	[62]
lincRNA-EPS	Mouse 4qC7	Promotes red blood cell maturation	[63]
T-ALL-R-LncR1	6q24.3	Induces apoptosis in T-ALL	[66]
LUNAR1 (Leukemia-Induced Noncoding Activator RNA)	15q26.3	Positive regulator of cell division	[67]
HOTAIR (Hox Transcript Antisense RNA)	12q13.13, encoded within the HOXC gene cluster	Oncogene, promotes chromatin relocalization	[68]
MALAT-1 (Metastasis Associated Lung Adenocarcinoma Transcript)	11q13.1	Regulates transcription and cell cycle	[69]
MEG3 (Maternally Expressed Gene 3)	14q32.2	Tumor suppressor	[70]
XIST (X-Inactive Specific Transcript)	Xq13.2	Regulates X chromosome inactivation during embryogenesis	[73]

Table 2.
Examples of lncRNAs involved in normal and malignant hematopoiesis.

Hu et al. [63] studied the lncRNA transcriptome of the erythroid lineage and uncovered numerous erythroid-specific lncRNAs that become induced during terminal differentiation of mouse fetal liver red blood cells in vivo. These authors showed that lincRNA-EPS (erythroid prosurvival) acts to promote red blood cell maturation by downregulating proapoptotic pathways. Knockdown of lincRNA-EPS severely compromised terminal differentiation of erythroid progenitors and resulted in elevated apoptosis. Conversely, its ectopic expression protected erythroid progenitors from apoptosis triggered by erythropoietin starvation. Functional studies indicated that lincRNA-EPS acts by repressing a number of proapoptotic proteins, most prominently the caspase-activating adaptor protein Pycard [63].

Recent studies have also provided evidence for the importance of several lncRNAs in immune cell function. For example, lncRNA NeST (Nettoie Salmonella pas Theiler's, cleanup Salmonella not Theiler's), also named Tmevpg1, modulates the ability of mice to respond to viral and bacterial infections. NeST is specifically expressed by the T_H1 subset of helper T cells. The expression of NeST regulates the degree of inflammation induced by infecting pathogens, such as Thelier's virus or Salmonella [64]. lncRNA-Cox2 (cyclooxygenase 2) acts during inflammatory signaling by modulating the expression of several immune response genes via interactions with regulatory complexes [65].

4.2 lncRNAs in malignant hematopoiesis

lncRNAs not only participate in normal hematopoiesis but also contribute to the pathogenesis of hematologic malignancies, representing a new class of potential biomarkers and therapeutic targets. These RNAs have significantly different expression levels in primary tumors and metastases, functioning both as oncogenes or as tumor suppressors. Some cancer-related lncRNAs could affect the development and progression of tumor by means of p53, polycomb repressive complex 2 (PRC2), and other signaling pathways. Others are not observed in normal tissue but are detected in cancer. For example, T-ALL-R-lncR1 appears to induce (together with protease-activated receptor 4 (PAR-4)) cellular apoptosis in T cell acute lymphoblastic leukemia cells (T-ALL) [66]. LUNAR1 (leukemia-induced noncoding activator RNA) is highly expressed in T-ALL cells, and its expression is dependent on signaling through the oncogenic NOTCH1 receptor [67]. lncRNAs HOTAIR (homeobox transcript antisense RNA) and MALAT-1 (metastasis-associated lung adenocarcinoma transcript) are associated with metastasis and recurrence [68, 69].

4.2.1 lncRNA deregulation in MDS

The first study describing lncRNA in the context of MDS was published in 2010 by Benetatos et al. [70] who studied lncRNA MEG3 (maternally expressed gene 3). Abnormal methylation of its promoter was observed in a third of MDS patients and in half of AML patients [70]. MEG3 was the first lncRNA described to have a tumor suppressor function. MEG3 is expressed in many human normal tissues, and numerous studies have demonstrated that its expression level is lost in various cancers. Low expression of MEG3 is associated with an increased risk of metastasis and poor prognosis in cancer patients [71, 72].

In 2013, Yildirim et al. [73] conditionally deleted lncRNA XIST (X-inactive specific transcript) in mice hematopoietic cells. XIST is perhaps the most well-understood lncRNA to date. This lncRNA is located on the X chromosome and is required for X chromosome inactivation during embryogenesis. Yildirim et al. [73]

demonstrated that mutant females developed a highly aggressive myeloproliferative neoplasm and MDS (mixed MPN/MDS) with 100% penetrance.

The first study examining the deregulation of lncRNAs on a genome-wide level in MDS was published in 2017 [74]. The authors combined NGS and microarray data in $CD34^+$ BM cells and identified several lncRNAs (linc-ARFIP1-4, linc-TAAR9-1, lincC2orf85, linc-RNFT2-1, and linc-RPIA) deregulated in MDS-EB2. In the same year, Yao et al. [75] profiled lncRNA expressions in 176 adult patients with primary MDS and identified four lncRNAs (TC07000551.hg.1, TC08000489.hg.1, TC02004770.hg.1, and TC03000701) with expression levels significantly associated with overall survival. Subsequently, the authors constructed a risk-scoring system with the weighted sum of these four lncRNAs. Higher lncRNA scores were associated with higher marrow blast percentages, higher-risk subtypes of MDS, complex cytogenetic changes, and mutations in RUNX1, ASXL1, TP53, SRSF2, and ZRSR2, whereas they were inversely correlated with the SF3B1 mutation. Patients with higher lncRNA scores had significantly shorter overall survival and higher 5-year leukemic transformation rate than did those with lower scores [75].

Although increasing numbers of deregulated lncRNAs are currently being described in MDS, only a few have been functionally characterized so far. Transcriptomic data may be used to construct network modules consisting of lncRNAs and protein-coding genes to enable functional analysis of lncRNAs with unknown functions. These networks are subsequently linked with annotated signaling pathways and gene ontologies. The resulting outputs provide a degree of functional annotation for differentially expressed lncRNAs in the disease and their potential roles in pathophysiology [74].

5. Other types of noncoding RNAs in MDS

Only very limited information regarding other groups of ncRNAs have been published for MDS to date. However, we can anticipate that introduction of next-generation sequencing of RNAs (so-called RNA-seq) will bring to MDS research many novel insights regarding various ncRNAs in the near future. This technology enables sensitive global detection of various RNAs across an unparalleled dynamic range. Particularly, small RNA-seq is predominantly used for detection of miRNAs. However, during library preparation, small RNAs are selected by electrophoresis with sizes typically ranging from 20 to 50 nt. This range of size selection allows for the capture of many other species of small RNAs in addition to miRNAs.

5.1 Piwi-interacting RNAs

In 2011, Beck et al. [76] conducted one of the early studies to apply small RNA-seq in MDS. These authors compared expression of small RNAs between low-grade (refractory anemia, RA) and high-grade (MDS-EB2) MDS patients and demonstrated the first evidence of piwi (P-element-Induced Wimpy Testis)-interacting RNAs (piRNAs) in MDS BM cells and their particular enrichment in low-grade MDS. PiRNAs are a relatively newly defined class of small ncRNAs with lengths from 26 to 32 nt. These RNAs lack sequence conservation and are more complex than miRNAs. PiRNAs have been linked to both epigenetic and posttranscriptional gene silencing of retrotransposons and other genetic elements in germline cells, particularly those involved in spermatogenesis.

Transcription of particular piwi proteins (*piwil1* and *piwil2*) that are required for the accumulation of piRNAs was also significantly upregulated in RA [76]. Recent studies indicated that the piwi-piRNA complex may have a role in

posttranscriptional silencing of damaged DNA fragments and that interrupting piwi-piRNA formation can lead to DNA double-strand breaks [77]. In summary, the study from Beck et al. [76] suggested that the enrichment of piRNAs in low-grade MDS may potentially protect DNA from the accumulation of mutations, a mechanism not observed in high-grade MDS. Moreover, they proposed that piRNAs might be used as diagnostic markers for low-grade MDS; however, further studies of piRNA roles in MDS pathogenesis are warranted.

5.2 Transfer RNAs

The abovementioned pioneer study from Beck et al. [76] also provided an early insight into the deregulation of tRNAs in MDS. The authors showed that ratios of tRNA to rRNA were significantly higher in MDS-EB2 compared to those of RA and controls. Because tRNAs are building blocks for protein synthesis and are required during translation, this may indicate an increased regulation of translation at this disease stage. Interestingly, a significant increase of tRNAs in tumor samples was reported by Pavon-Eternod et al. [78] In addition, tRNAs have been shown to inhibit cytochrome c-activated apoptosis [79]. Taken together, Beck et al. [76] hypothesized that high tRNA content seen in EB2 may contribute to the two well-known characteristics of high-grade MDS, decreased apoptosis, and high rate of leukemic transformation.

Guo et al. [80] performed small RNA-seq in paired pre- and posttreatment samples from MDS patients receiving therapy with HMAs. In the sequencing data, the number of reads aligned to tRNA-derived small RNAs (tDRs) (78.81%) vastly outnumbered those aligning to miRNAs (4.43% of reads). The tRNA fragments that were captured by miRNA-seq might be a result of either active cleavage or artifacts of the miRNA-seq library construction. The authors identified six tDR fragments that were differentially expressed between MDS and normal samples. Three tDRs demonstrated increased expression in MDS (chrM.tRNA10.TC, chr12.tRNA8.AlaTGC, and chr16.tRNA4.ProAGG), while three were decreased (chr1.tRNA58-LeuCAA, chr19.tRNA8-SeC(e)TCA (SeC(e)TCA), and chr19.tRNA4-ThrAGT). Moreover, they identified a panel of four tRNA fragments (chr6.tRNA157.ValCAC, chr11.tRNA17.ValTAC, chrM.tRNA12.TS1, and chrX.tRNA4.ValTAC), whose combined expression in the pretreatment samples together was predictive of the likelihood of response. Deeper focus on mitochondrial tRNAs revealed that MT-TS1 (mitochondrially encoded tRNA serine 1) was the only mitochondrial tRNA to have a significant association with treatment response [80].

6. Conclusions

The discovery of ncRNAs has initiated a new era in molecular biology, completely changing our view of “junk” DNA that it is no longer considered unnecessary ballast. Mouse models have clearly demonstrated key functions of ncRNAs in regulatory networks and their ability to significantly influence biological processes. In the hematopoietic system, ncRNAs represent important regulators of HSC “stemness” and differentiation; therefore, it is not surprising that deregulation of ncRNAs also occurs in MDS.

Currently, we possess comprehensive information regarding the impact of miRNA deregulation on the pathogenesis of MDS. The efforts of current research activities aim to apply these findings to clinical practice, testing the potential diagnostic/prognostic value of selected miRNAs for MDS. However, miRNAs also represent promising therapeutic agents or targets. miRNA-based drugs are designed to

reduce the expression of oncogenic miRNAs or, conversely, to increase levels of miRNAs with tumor suppressor functions. Unlike targeted inhibition or activation of a single protein-coding gene, the administration of miRNA antagonists or their mimics may potentially improve the desired effects, as these molecules can regulate several genes, often in specific signaling pathways implicated in tumorigenesis. Several pharmaceutical companies already have miRNA therapeutics in their developmental pipelines [81]. In 2012, the first cancer-targeted miRNA drug, MRX34 (a liposome-based miR-34 mimic), entered phase I clinical trials in patients with advanced hepatocellular carcinoma, and this mimic has attracted considerable attention from both academic researchers and pharmaceutical companies [82]. MRG-106, a synthetic antagonist of miRNA-155, is currently being tested by MIRagen Therapeutics in patients with cutaneous T-cell lymphoma [83]. However, testing miRNAs as potential therapeutic agents or targets in MDS therapy still requires initial exploration in in vitro models before evolving to future clinical trials.

Information regarding the contribution of other categories of ncRNAs, including lncRNAs, to the pathogenesis of MDS remains scarce. However, given the large number of ncRNAs encoded in the human genome and the complexity of their interactions, it can be expected that, in the near future, we will reveal a number of ncRNAs involved in MDS. It can also be anticipated that we will identify new predictive markers of progression and responses to therapy among these molecules.

To conclude, the diagnostic and therapeutic possibilities of ncRNAs undoubtedly have profound potential in MDS. However, although the effects of some miRNAs have already been demonstrated, it is certain that the importance of ncRNAs in MDS will be fully understood only in the future and that many years of research and clinical trials remain before the eventual application of ncRNAs in clinical practice to classify, monitor, and treat this disease.

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Conflict of interests

The authors declare that they have no competing interests.

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